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AT GREENSBORO

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HONORS PAPERS

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CONTENTS

SENIOR HONORS THESIS:

PERSONAL PROBLEMS IN CREATIVITY

by

Margaret Lynne Ainsley

Submitted as an Honors Project
in the
Department of Art

The University of North Carolina
at Greensboro
1969

Approved by

Director

Examining Committee

James Applewhite

Wm Dabney

A sequence of paintings, represented by photographs and poems and notes, constitutes the Senior Honors Thesis of Margaret Lynne Ainsley for the year 1968-69. Both the work presented and that which is absent (including visual forms not yet materialized) represent a search for direction -- in a personal development and expression of painting, and in an analysis of purpose relative to the person.

The thesis is an open-ended process -- a medium of growth and change congruent with the changing state of a personal philosophy. The result, therefore, is not the immediate production of a climactic work or works, but the amalgamation of many small results -- successes and failures leading to a clearer recognition of those statements which are strongest and hence, to direction in limiting and exploring the possibilities thus set up. It is ended, but not completed...

The representations, mounted to resemble a splice of film with sound track, have been deposited in the library.

THE PROCESS OF GEOGRAPHICAL SUBSPECIATION AND EVIDENCE FOR ITS OCCURRENCE IN STRAINS OF THE GENUS Neurospora

by
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Table of Contents

The problem of the origin of species, more properly of "the splitting of an originally uniform species into several daughter species," (Mayr, 1963, p. 426) is one of the fundamental concerns of biology. Directly related to it are the principles of evolution, genetics, and ecology. Much of the recent literature in biology deals with species concepts and with the operant isolating mechanisms in various species. Dobzhansky (1951) has reviewed extensive work with Drosophila relative to the general principles of speciation. Mayr's classical work on evolution and species in general, Animal Species and Evolution, 1963, is well known.

The Ascomycete Neurospora has been chosen as an experimental organism to investigate the occurrence of subspeciation for several reasons. It is ideal because of the relative technical ease with which laboratory cultures can be handled and its extensive use in experimental genetics. Also, strains of Neurospora from widespread geographical areas were easily available. Morten Lange, in The Fungi, Vol. III, (1968) cites indefiniteness of the species concept in fungi and the lack of information from genetic studies using hybrids at the specific level. Srb (1959) reported studies involving crosses between strains of Neurospora from different geographical areas, but his emphasis was on demonstrating the cytoplasmic nature of the transmission of the Slow Growth trait in N. crassa.

The following is an account of an attempt to determine the relationships of several strains of Neurospora of different geographical origin to standard U. S. laboratory wild-type stocks. The criterion for species classification was the fertility of a cross between each exotic strain and a domestic laboratory strain. Unusual growth

patterns, viability, and morphology and general appearance of the meiospores produced by each cross were tabulated and compared. Since this study involves only one or a few individuals from each area, these are assumed to be representative of their respective populations. Only to the extent that each is typical of its population are these individual events significant in determining species status.

Materials and Methods

Several strains of Neurospora from different areas were obtained through the Fungal Genetics Stock Center at Dartmouth College, Hanover, New Hampshire. Those used in the experiment were:

Origin	Mating Type	FGSC No.
Costa Rica	A	851
Costa Rica	A	852
Fiji	A	435
Fiji	a	432
Liberia	a	967
Liberia	A	961
? (LEIN)	A	847
Java	a	431
Liberia 4	A	434
North Africa I	A	430
New Zealand S-3	a	644
New Zealand S-4	a	642
New Zealand M-1	a	643
Philippine Islands 4	a	433
Puerto Rico 18	a	429
Singapore 2	a	436

The standard laboratory wild-types used were 74-OR23-1A for parent of mating type "A" and Emerson #692 for mating type "a".

Crosses were made on slants of Perkins Synthetic Cross Medium by inoculation of slants with each exotic strain and fertilization by adding conidia from the standard strain after six days incubation at 25°C. Mycelial growth patterns, conidiation, and protoperithecial formation were observed and recorded for each exotic strain during its growth before fertilization. Reciprocal crosses using the standard strains as perithecial parents were made concurrently to reveal any cytoplasmic sterility factors which might be operating.

After maturation, the crosses were tabulated as to morphological characteristics of the perithecial fruiting bodies and the extent to which the ripened spores were shot from the perithecia. Variatiations in spore shape, size, number, and color were recorded.

The major criterion by which fertility was measured was viability of the spores produced by the crosses. Several hundred random spores from each cross were spread on plates of complete agar medium, were heat-shocked 15 minutes at 60°C, and incubated at approximately 9°C for 24-48 hours. Then by use of a dissecting microscope, germination percentages were obtained by counting the number of spores on each plate which had begun to put forth germination tubes.

Results

The major criterion for postulating that species divergence is in progress between the standard U.S. laboratory cultures and any exotic strain was percentage germination of the ascospores produced by the cross. In each case where spores were numerous enough, germination percentages were based on testing 300 to 500 spores. These results are shown in the following table.

Germination Percentages of Ascospores

Exotic	Crossed to	Origin	Germ.
429 a	74-0R23-1A	Puerto Rico	89%
851 A	Em. #692 a	Costa Rica	76%
430 A	Em. #692 a	No. Africa	74%
431 a	74-0R23-1A	Java	0%
852 A	Em. #692 a	Costa Rica	47%
436 a	74-0R23-1A	Singapore	40%
967 a	74-0R23-1A	Liberia	18%
432 A	Em. #692 a	Fiji	0%
847 A	Em. #692 a	? (LEIN)	65%
434 A	Em. #692 a	Liberia	0%
435 A	Em. #692 a	Fiji	NC
961 A	Em. #692 a	Liberia	NC
644 a	74-0R23-1A	New Zealand	NC
642 a	74-0R23-1A	New Zealand	NC
643 a	74-0R23-1A	New Zealand	NC
433 a	74-0R 23-1A	Phillipine Isl.	NC

NC -- No Cross: no ascospores formed

Generally, the fertility of the standard wild types appears to be greater in crosses with other New World strains, and to drop off noticibly with increasing geographical remoteness to the strain with which they are crossed. The least success was obtained with crosses involving strains from the Far East and New Zealand. However, in view of the many complex variables which can conceivably affect the crosses, it seemed advisable to analyse several other characteristics of the crosses.

Early in the cross, notations were made of the different growth patterns of the protoperithecial exotics. Somewhat later, extent of conidia formation, as well as relative number and size of protoperithecia were noted. Several strains appeared to grow well vegetatively, producing relatively normal looking mycelia and healthy amounts of conidia. However, no fruiting bodies, or very small ones developed. When germination results of these crosses were compared, it was found that they produced no ascospores. In these cases it seems probable that crossing was prevented by the factors which hindered protoperithecial formation.

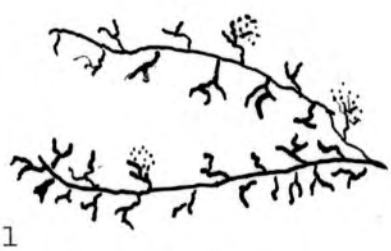
Another instance of cross failure was reflected in the results of several crosses in which the protoperithecial exotic grew in a generally unhealthy manner, exhibiting slow growth, poor conidiation, a thin mycelium, and few protoperithecia. This condition seems to reflect at least a partial nutritional requirement not being met by the minimal cross medium. Indeed, strain #967, the clearest example of this type of growth is reported by FGSC to respond to threonine.

There were, however, several crosses with no spores or with very low germination in which the protoperithecial exotic grew normally, produced numerous aerial conidia, and formed adequate numbers of protoperithecia which were of normal size. In these cases it is postulated that crossing was prevented by genetic differences in the strains.

Sketches of Vegetative Growth of Some Strains which Did Not Produce Protoperithecia

435
Modified Branching

643
Thin Growth



961
Crinkled Mycelium

644
Reduced Branching

Normal Mycelium

Magnification 500 X.

Observations at 3 Days Growth Compared to Cross Failure

Strain	Conidia	Mycelium	Protoperithecia No.	Size	Germ.
premating interference:					
435	Normal	Thick	None	--	NC
961	Normal	Thick	None	--	NC
643	Normal	Thick	Few	Very Small	NC
644	Normal	Thick	None	--	NC
vegetative interference:					
967	None	Slow	Few	Small	18%
postmating interference:					
432	Normal	Thick	Average	Average	0%
431	Normal	Thick	Average	Average	0%
434	Normal	Thick	Average	Small	0%

Spore Characteristics and Germination

Strain	Shooting	Size	Number	Relative # white	Shape	Germ.
852 A	+++	+	++	+	irregular	47%
851 A	++++	++	++	++	regular	76%
432 A	-	++	+-	++++	irregular	0%
435 A			No spores			NC
967 a	++	++	++++	+	very irregular	18%
961 A			No spores			NC
431 a	++	++	++	++	irregular	65%
434 A	-	+	+	++++	irregular	0 %
430 A	-	+++	++	++	irregular	74%
644 a			No spores			NC
643 a			No spores			NC
642 a			No spores			NC
433 a			No spores			NC
429 a	++++	++	++++	-+	irregular	89%
436 a	-	+	++	+++	irregular	40%

Another comparison that was made was between viability of ascospores and various characteristics of the spores themselves. Here, considerable resemblances were found. In general, those crosses showing low germination rates produced large numbers of white spores, many small and irregularly shaped spores, but small total numbers of spores. Viability also correlated well with the degree of expulsion of spores from the mature perithecia.

Discussion

Most writers agree that morphological classification alone does not reflect the actual divisions of nature into kind, and that the accepted definition of a species is a group of reproductively distinct organisms which share genetically similar characteristics. This comment by Hamilton (1967) illustrates the concept very well:

> A species may be envisioned as an isolated pool of genes flowing through space and time, constantly adapting to changes in its environment as well as to the new environments encountered by its extension into other geographic regions. (p. 76)

The emphasis here on the continuity of the species through space and time and on the change which occurs within the species in time should be noted. Another point included in this definition is the role played by environment in the change within a species by the operation of natural selection.

Mayr (1963) warns against reacting to the quandary over classification with the observation that such is merely the arbitrary and artificial product of the mind of man and imposes imaginary boundaries which do not exist in nature. To think thus, he says, is to become blinded to one of the most real and fascinating problems which biology presents--that of reproductive isolation and its mechanisms.

Yet another important genetic characteristic of a species is variation--that is, the fact that its members are different both in genetic content and in overt expression of that content. This area of genetics has been widely studied with regard to many organisms. The presence of differences among the individuals of a population has long been known, and many examples among both plants and animals have been recorded. Dobzhansky (1951) studied this phenomenon in Drosophila and reported on the role in variation played by chromosomal inversions. There are numerous examples of variation in plants, which are strongly limited to locality and place a premium on adaptedness to the environment.

Several examples of physiological variation have been found in Neurospora. Three forms of aryl-B-glucosidase have been found among different Neurospora strains (Mahadevan and Eberhart, 1962). Two kinds of tyrosinase have also been found (Horowitz and Fling, 1953). There is evidence of differences in inducible cellulase activity among the strains used for crossing in this experiment (Beck and Eberhart, unpublished work).

There are two primary sources of variation. These are (1) mutation and (2) recombination. The former in natural populations is a random, spontaneous process and may be of a very small order, or of a larger kind affecting the entire chromosome. Recombination is also of two types. The first arises during meiosis (crossing over and segregation) and produces new kinds of gametes. The second arises from the joining of gametes to form new combinations of gene homologies. Both sources of variation operate in sexually reproducing populations of a species. (Hamilton, 1967, p. 13)

Among the most striking examples of variation are the numerous instances of geographic variation of populations of a species. These range from substrate adaptation, or

preference, to length of diapause in insect species. Mayr (1963, p. 333) states that these and other geographic variations as a whole are adaptive. Not only is variation adaptive, but such adaptation and evolutionary change are considered to be two sides of the same coin--"the continuous adjustment of an integrated gene complex to a changing environment" (Mayr, 1963, p. 332).

Natural selection is a well-known topic, and one which has recieved much attention in writings on evolution. G. G. Simpson defined selection "as anything tending to produce systematic, heritable change in populations between one generation and the next" (Hamilton, 1967, p. 51). Many writers tend to de-emphasize the elimination effect of selection on nonadaptive characters which Darwin and others expounded. However, Mayr (1963, p. 182) reminds us that this "elimination of excess genetic variability" is a very important stabilizing mechanism in the changes which take place in natural populations. Still the principle consideration concerning natural selection is its editing effect upon the genetic content of the next generation. This fact is stated thus by Mayr (1963, p. 183):

> let it be clearly understood that what really counts in evolution is not survival but the contribution made by a genotype to the gene pool of the next and subsequent generations.

Thus, natural selection has two aspects which are inseparable in operation: its negative, or stabilizing, function; and its positive, or direction-giving one.

The question, "what happens when a new species is formed?" must be answered by interaction between the characteristics of a species and the forces acting upon its genetic development. It is postulated that geographical isolation of populations produces physical barriers to gene flow between them. The adaptive response of the gene pool of the local population to selection pressure

of the environment results eventually in genetic divergence so great that gene flow between them becomes impossible. This constitutes reproductive isolation and is the definitive aspect of species. Mayr (1963) discusses these processes as probably the most important phenomena of speciation. There are still some geneticists who believe that the accumulation of differences by populations is merely incidental to species differentiation (Love, 1965, p. 409-410). However, Mayr's theory seems to be more widely accepted, as well as to better coincide with empirical evidence. He (1963, p. 554) also warns against assuming that geographic isolation always leads to speciation. A successful new species is a rare occurrence, whereas geographical isolates are up to 100 times as common. Therefore, spatial isolation, variability within the gene pool, and natural selection play key roles in the "multiplication" of species.

The biological properties of individuals which prevent inter-crossing between populations are termed isolating mechanisms. Mayr (1963) divides these into two categories: premating mechanisms and postmating mechanisms. Included in premating mechanisms are ecological factors of the populations, such as habitat adaptation, an important mechanism in plants, and differences in the reproductive structures which prevent the successful contact of gametes. Postmating mechanisms include those "barriers which prevent successful hybridization", such as gametic mortality, zygotic mortality, hybrid inferiority, and hybrid sterility. Ecological barriers are often the primary and most effective ones, since many species can interbreed, but do not normally in nature. According to Mayr, isolating mechanisns arise as by-products of genetic divergence of isolated populations. He postulates that the first such mechanism is often cross sterility acquired in geographical isolation. Upon

contact, the cross sterile groups become subject to selection pressure to prevent wastage of gametes by acquisition of additional isolating mechanisms. This, along with sufficient niche divergence to permit ecological compatibility, permit the two groups to coexist while each maintains the integrity of its genetic system.

In order to discuss the nature of isolating mechanisms, it is necessary to consider a point which Mayr (1963) repeatedly reiterates. That is the complexity and integration involved in the genetic systems of species. The tendency of genes to interact with each other is termed cohesion. Polygeny, the influence of several genes upon one character, and pleitropy, the production of multiple effects by a given gene, are documented examples of such interaction. The success of a given gene will depend at least partly upon how compatible it is with the other members of the gene pool. Mayr (1963, p. 296) describes this interaction in this way:

> The result of the close interdependence of all genes in a gene pool is tight cohesion. No gene frequency can be changed, nor any gene be added to the gene pool, without an effect on the genotype as a whole, and thus indirectly on the selective value of other genes.

Isolating mechanisms, then, must be considered the products of the genetic system as a whole. They are a defense against the destruction of its cohesion.

Complex genetic differences are ultimately attributable to differences in DNA base sequence. There has been considerable use of cytological and biochemical methods in studies of species relationships. Jukes (1966) states that while analysis of comparative guanine plus cytosine content provides limited usefulness, experiments involving annealing of separated DNA from two species provide the best direct evidence of the homology of the base sequences of their DNA--and thus of the closeness of their evolutionary

relationship.[1] Furthermore, the greater degree of cross combination of DNA from closely related species is explained by postulating groups of similar regions, implying the preservation of gene complexes rather than of scattered single genes during evolutionary change.

Another aspect of biological isolation which would fall under postmating mechanisms involves extent of chromosomal pairing. Dobzhansky's (1951) work with Drosophila stressed the importance of inversions in preventing pairing between homologous chromosomes. Other gross structural aberrations in chromosomes might also be expected to weaken pairing forces. From work with interspecific crosses in wheat, Riley and Law (1965) concluded that:

> the amount of pairing is regulated by the integrated operation of numerous genes, each producing an individually small effect, and of major genes with more profound manifestations. Moreover, there are pronounced nonallelic interactions between the separate components of the system... (p. 78)

It is also pointed out (Riley and Law, 1965, p. 81) that mere base sequence correspondence, which must be repetitious in many areas throughout the chromosome, cannot completely account for strictly homologous pairing as it is observed. This would indicate a genetic system to regulate synapsis.

Another isolating mechanism has been observed in some species (Mayr, 1963, p. 42). This is a cytoplasmic factor producing interspecific sterility. The presence

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[1]Annealing, as described by Jukes, is the process of heating extracted DNA from the two sources, which effects separation of the strands. Upon combination of the two DNA's and slow cooling, the degree with which they interchange partners indicates the degree of correspondence of base sequences. This can be determined by isotopic labeling of DNA from one source, and detection of the degree of its entrapment by the other DNA implanted in an agar surface.

of such a factor can be detected by reciprocal crosses using males of each species crossed to females of each.

In summary, the characteristics of a species include biological reproductive isolation, wide genetic variation among its members, and adaptedness to the environment. Geographical variation between populations of a species, when spatial isolation occurs, provide the genetic basis from which a new species can arise in response to selection pressures of a changing environment. If adaptive divergence of an isolated population proceeds far enough to introduce a biological barrier to interbreeding (often cross sterility) with former conspecific populations, subsequent contact between them may cause them to become subject to selection for additional isolating mechanisms. This eventually results in new species. Completed speciation is a comparatively rare occurrence, although geographic isolates are common, and geographical speciation the most highly credited theory of speciation (Mayr, 1963).

An important aspect of gene pools is cohesion, or tendency toward interaction which affects every gene, and is affected by each. Thus, the genetics of speciation are not simple. Consideration of the complex, interdependent nature of gene action is necessary in understanding the speciation process.

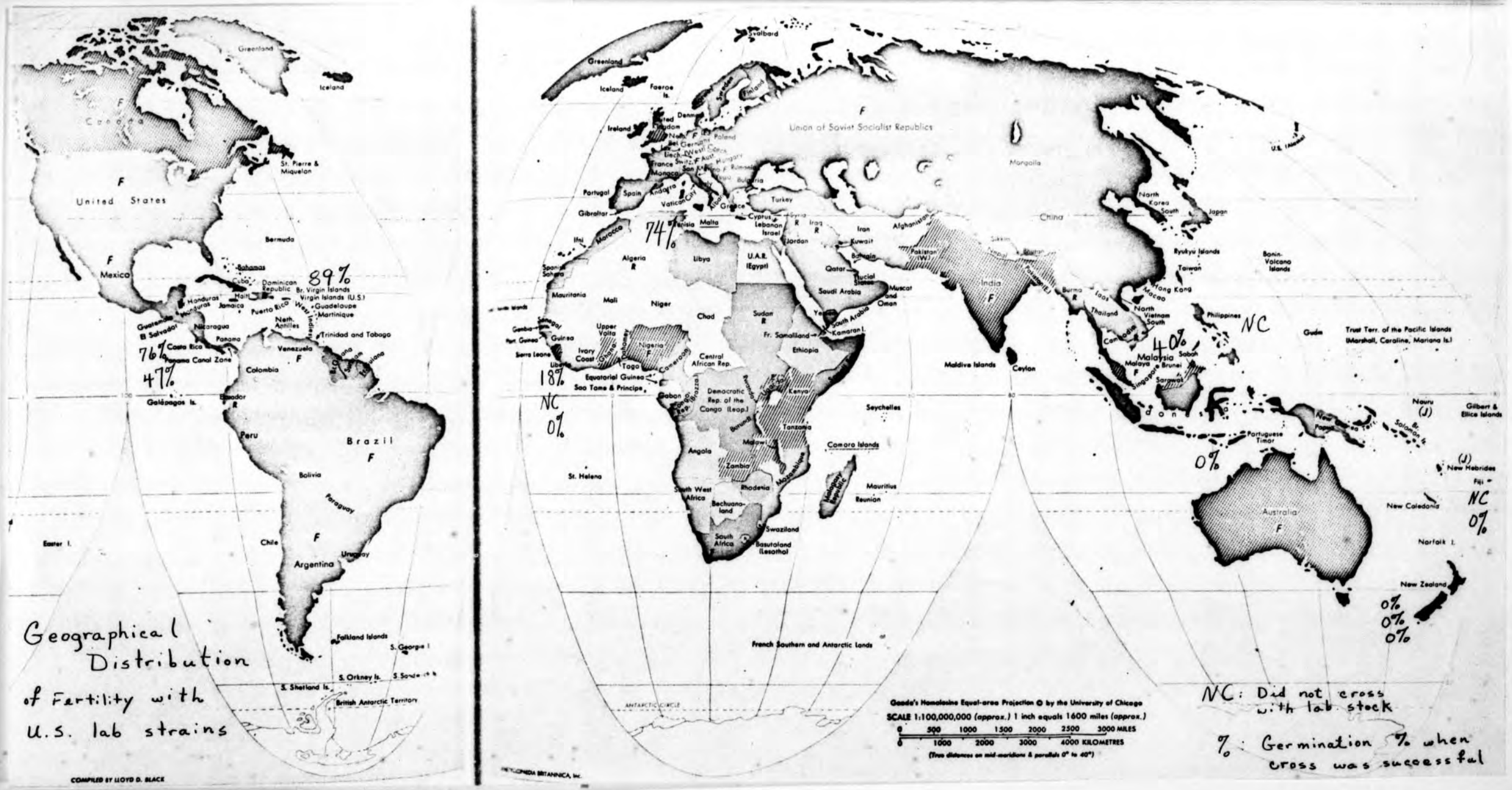
Conclusion

From results of the crosses made, it is apparent that there does exist some degree of reproductive isolation between several of the foreign strains and the standard laboratory strains. Caution should be used in interpreting these results, since they represent individual strains and not a wide sampling of the populations. A cross between the two laboratory strains was not made, and germination percentage of such a cross was assumed to be at least 90%.

Strains with the lowest fertility seem to be separated by the greatest distances. It is impossible to determine from this data what isolating mechanisms are operating, although it would seem that in several instances (435, 961, 643, 644) they are premating, while in others (432, 431, 434) they are postmating. It is likely that cross-sterility is at least one barrier in operation, but there may be others as well. There are varying degrees of reproductive isolation evident from the widespread differences in fertility. Striking differences in vegetative growth, conidiation, and physiology exemplify geographic variation. It is known that this is an element of the geographic speciation process.

Further crosses between each exotic strain and its neighbors might reveal more clearly the degree of specific differences among them. More extensive analysis of progeny, comparing for example backcrosses to each parent might also prove interesting. Cytological analysis of the chromosomes of the strains might also provide information on chromosomal aberrations.

The chief value of this study lies in the areas of possible investigation which it brings to light. The existence of such wide variation among members of the same "species" may be used as a basis for genetic studies of the mechanisms of reproductive isolation and speciation.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Zambia
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St. Helena
South West Africa
Rhodesia
Bechuanaland
South Africa
Swaziland
Basutoland (Lesotho)
French Southern and Antarctic Lands
ANTARCTIC CIRCLE
74%
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Geographical Distribution of Fertility with U.S. lab strains
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Goode's Homalosine Equal-area Projection © by the University of Chicago
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0 500 1000 1500 2000 2500 3000 MILES
0 1000 2000 3000 4000 KILOMETRES
(True distances on mid-meridians & parallels 0° to 40°)
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NC: Did not cross with lab stock
%: Germination % when cross was successful

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